




LETTER

Combined exposure to CO₂ and H₂S significantly reduces the performance of the Mediterranean seagrass *Posidonia oceanica*: Evidence from a volcanic ventGeraldina Signa,^{1,2} Valentina Scutтери,³ Agostino Tomasello ^{1,2*} Valentina Costa ⁴ Silvia Casabianca,^{2,5} Giovanna Cilluffo,^{1,2} Cristina Andolina,^{1,2} Salvatrice Vizzini ^{1,2}

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Scientific Significance Statement

Seagrasses are expected to thrive in future acidified oceans due to their ability to overcome the low CO₂ diffusion into plant tissues. However, the effects of the co-occurrence of CO₂ and toxic compounds on the plant and, as a consequence, on the structure and function of the entire ecosystem are largely unknown. In this study, we show that the co-occurrence of CO₂ and H₂S in a shallow volcanic vent has detrimental effects on the seagrass *Posidonia oceanica*, from the leaf to the meadow level, due to sulfide intrusion, which impairs growth performance. The expected beneficial effects of high CO₂ levels on seagrasses may be dampened by other factors, highlighting the need to consider the natural complexity of ecosystems in ocean acidification studies.

Abstract

Although seagrasses are expected to thrive in future acidified oceans by overcoming low CO₂ diffusion into plant tissues, the co-occurrence of environmental stressors may affect their growth. Volcanic CO₂ vents are often associated with toxic gases and metal-rich fluids representing ideal sites to assess the effects of multiple stressors. We evaluated the response of *Posidonia oceanica* growing near shallow CO₂ vents characterized by H₂S spill-out by comparing meadow structure and phenology to an area with no gas emissions. Seagrass descriptors at meadow, shoot and leaf level indicated that *P. oceanica* experienced stressful conditions at the vent area, in clear contrast to the flourishing features of *P. oceanica* previously described at CO₂ vents with no evidence of

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Geraldina Signa and Valentina Scutтери share co-first authorship.

Additional Supporting Information may be found in the online version of this article.

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toxic inputs. Furthermore, the reduction in both leaf $\delta^{34}\text{S}$ and growth at the vent area indicates that sulfide intrusion occurs and affects seagrass growth performance, dampening the expected beneficial effects of high CO₂ levels.

Anthropogenic CO₂ emissions alter the carbon chemistry of the oceans by increasing $p\text{CO}_2$ and decreasing pH, a phenomenon known as ocean acidification (OA), which can alter the stability and functioning of marine ecosystems (Mostofa et al. 2016; Sunday et al. 2017; Cattano et al. 2018; Hall-Spencer and Harvey 2019; Aiuppa et al. 2021). Natural CO₂ vents are subtidal marine areas characterized by the emission of volcanic gases (mainly CO₂) that cause the formation of spatial pH gradients and create the conditions predicted by future acidification scenarios (i.e., pH decrease of 0.2/0.4 units by 2050–2100 according to the RCP8.5 scenario; Bindoff et al. 2022). They are therefore recognized as natural laboratories where scientific hypotheses on OA can be tested in situ (Hall-Spencer et al. 2008; Vizzini et al. 2017; Foo et al. 2018; Rastrick et al. 2018; Mirasole et al. 2020). Moreover, CO₂ vents are distributed across temperate and tropical latitudes overcoming the inherent limitations of laboratory and mesocosm studies by encompassing the environmental variability of natural systems. Although CO₂ accounts for the majority (90–99%) of volcanic emissions (Aiuppa et al. 2021), it is often associated with other gases (i.e., mainly N₂, O₂, CH₄, H₂S) and metals, which may represent non-negligible additional stressors and confounding factors when studying the ecological effects of OA (Vizzini et al. 2013; Agostini et al. 2015; Zitoun et al. 2020). Such a combination of chemical stressors may create extreme environmental conditions to which dwelling species must adapt in order to survive and thrive, and therefore provides opportunities to study their physiological, morphological, and behavioral responses and adaptations to future climate change conditions.

Seagrasses are habitat-forming species that play a crucial role in temperate and tropical coastal zones, supporting high biodiversity and delivering multiple ecosystem services (Fourqurean et al. 2012; Ondiviela et al. 2014). Despite some species-specificity (Russell et al. 2013; Ow et al. 2015), it is generally accepted that seagrasses, along with other primary producers, will be the “winners” in a high-CO₂ world because of their strong affinity for dissolved inorganic carbon, mainly CO₂ and HCO₃⁻, which can stimulate primary production leading to increased photosynthetic rates and standing crop (resource-effect of CO₂) (Koch et al. 2013; Sunday et al. 2017). However, the combined exposure to high CO₂ and environmental stressors such as toxicants (Vizzini et al. 2013), increased temperature (Repolho et al. 2017; Collier et al. 2018) or eutrophication (Campbell and Fourqurean 2014) may result in antagonistic effects where the stressor negative effects outweigh the beneficial effects of OA (Repolho et al. 2017; Perry et al. 2019). In contrast, to our knowledge, the effects of combined exposure to high CO₂ and H₂S, one of the most potent phytotoxins in the marine environment (Lamers et al. 2013),

have never been assessed. There is evidence that sediment porewater sulfides can enter and diffuse into seagrass tissues, disrupting plant physiology and exerting toxic effects. For instance, sulfide intrusion can suppress photosynthesis in *Zostera marina* (Holmer et al. 2005) and leaf production and growth in *Posidonia oceanica*, ultimately affecting survival (Calleja et al. 2007; Frederiksen et al. 2007; Garcias-Bonet et al. 2008).

In this context, the stable sulfur isotope ratio ($\delta^{34}\text{S}$) is considered a valuable proxy for sulfide intrusion in seagrasses (Holmer and Hasler-Sheetal 2014). Potential sulfur sources for seagrasses have different isotopic signatures, with porewater sulfides being significantly negative ($\delta^{34}\text{S} = -10\text{‰}$ to -20‰) compared to seawater and porewater sulfates ($\delta^{34}\text{S} \approx +21$ and $+20\text{--}60\text{‰}$, respectively) (Frederiksen et al. 2008). These differences can therefore be used to trace the origin of sulfur in seagrass tissues. Thus, a decrease of *P. oceanica* $\delta^{34}\text{S}$ from its normal values toward less positive values indicates that sulfur from sedimentary sulfides has accumulated in plant tissues and negative effects on plant performance are expected (Holmer and Hasler-Sheetal 2014).

Here we aimed to investigate the response of structural traits of *P. oceanica*, the foundation seagrass species forming one of the most important coastal ecosystems in the Mediterranean Sea, to combined exposure to CO₂ and H₂S in a natural hydrothermal vent, considering multiple levels of biological organization (i.e., meadow, shoot, and leaf) of the seagrass living near and away from the shallow vent. Although it was not possible to test the effects of CO₂ and H₂S individually, we hypothesized that the stressor effect of H₂S would dampen the resource-effect of CO₂, so that the expected increase in density, biomass, and canopy height at the meadows near the vent compared to a reference area would not occur. We also investigated the effects of co-exposure to CO₂ and H₂S on leaf epiphyte biomass and grazing pressure, to evaluate the propagation of the seagrass response throughout the meadow ecosystem.

Materials and methods

The study was carried out east of Panarea Island (Aeolian Archipelago, southern Tyrrhenian Sea) in the submerged area enclosed by the islets of Dattilo, Lisca Bianca, Bottaro, and Lisca Nera. Here, shallow vents dominated by CO₂ (~90–99 vol.%), but also featured by N₂ and H₂S emissions (both ~0–6 vol.%), and pH gradients (Italiano and Nuccio 1991; Steinbrückner 2009; Romano et al. 2019) characterize the subtidal environment. White/yellowish deposits have been recorded around volcanic emissions due to sulfur precipitation

and bacterial mat formation by sulfur-oxidizing bacteria (Gugliandolo et al. 2006; Steinbrückner 2009). The area has also been characterized by parossistic events, such as in November 2002, when explosive submarine activities west of Bottaro Islet severely affected the surrounding environment and biota (Aliani et al. 2010; Vizzini et al. 2010). The annual rhizome elongation of the nearby *P. oceanica* collapsed over the next 2 yr (Vizzini et al. 2010), followed by a gradual improvement with a complete recovery by 2010 (Noè et al. 2020).

Fieldwork was carried out at two areas with similar depth (~ 9 m), sandy bottom, wind, and currents, and characterized by the presence of *P. oceanica* meadows. A venting area, hereafter referred to as “vent,” was identified east of Bottaro islet (38°38.270'N, 15°6.700'E), where continuous gas bubbling

with CO₂ (96–99%) and H₂S (0.5–2.5%) emissions was observed (Italiano and Nuccio 1991; Caracausi et al. 2005). A non-venting area, hereafter referred to as “reference,” was located east of Lisca Nera (38°38.110'N, 15°6.460'E) and Dattilo (38°38.370'N, 15°5.980'E) islets, where gas emissions and fluid discharges were never recorded (Italiano and Nuccio 1991; Tassi et al. 2014). Within each area, two sites with similar environmental characteristics were randomly selected. The first sampling campaign, in July 2010, aimed to investigate the seagrass response at the meadow level only: shoot density was recorded by SCUBA divers at each site (six replicates) using a quadrat metal frame (40 × 40 cm) (Buia et al. 2004). During the second sampling campaign, in July 2011, the investigation at the meadow level was repeated and

Table 1. Mean ± SD values of the *Posidonia oceanica* descriptors at the meadow (a) shoot (b), and leaf (c) levels measured at the vent and reference areas in 2010 and 2011. Leaf δ³⁴S is also shown (d).

Descriptor	Year	Area	
		Vent Mean ± SD	Reference Mean ± SD
(a) Meadow			
Density (shoot m ⁻²)	2010	261.5 ± 58.8	435.9 ± 128.8
	2011	248.4 ± 52.8	465.1 ± 104.4
(b) Shoots			
Total leaves (<i>n</i>) 2011		6.0 ± 2.2	3.9 ± 0.6
Adult leaves (<i>n</i>)		3.7 ± 1.6	2.9 ± 0.6
Intermediate leaves (<i>n</i>)		1.5 ± 0.5	1.0 ± 0.5
Juvenile leaves (<i>n</i>)		0.8 ± 0.6	0.0
Surface (cm ² shoot ⁻¹)		61.5 ± 23.7	243.0 ± 73.8
Biomass (mg dw shoot ⁻¹)		345.4 ± 146.8	1350.4 ± 476.6
Green tissue (%)		0.9 ± 0.0	1.0 ± 0.0
Brown tissue (%)		0.1 ± 0.0	0.0 ± 0.0
Leaf epiphyte biomass (mg dw shoot ⁻¹)		25.6 ± 11.3	172.0 ± 98.0
Coefficient A (%)		0.4 ± 0.2	0.7 ± 0.2
(c) Leaves			
<i>Adult leaves</i>			
Length (cm) 2011		22.7 ± 6.4	79.7 ± 23.7
Width (cm)		0.6 ± 0.1	0.8 ± 0.1
Biomass (mg dw leaf ⁻¹)		79.0 ± 28.2	382.4 ± 134.5
Sheath length (cm)		1.9 ± 0.6	4.4 ± 1.1
Sheath biomass (mg dw leaf ⁻¹)		15.5 ± 11.3	45.8 ± 27.1
Epiphyte biomass (mg dw leaf ⁻¹)		6.3 ± 3.8	53.0 ± 30.4
<i>Intermediate leaves</i>			
Length (cm)		14.8 ± 8.7	68.2 ± 26.7
Width (cm)		0.6 ± 0.1	0.8 ± 0.1
Biomass (mg dw leaf ⁻¹)		35.4 ± 22.1	241.5 ± 122.9
Epiphyte biomass (mg dw leaf ⁻¹)		1.5 ± 2.4	18.3 ± 14.7
(d) Stable isotopes			
Leaf δ ³⁴ S (‰) 2011		10.6 ± 0.8	15.5 ± 0.5

extended to the shoot and leaf level: five shoots were randomly collected at each site for the assessment of shoot and leaf descriptors (Table 1, Buia et al. 2004) including the analysis of stable sulfur isotopes ($\delta^{34}\text{S}$) of *P. oceanica* leaves and the associated epiphyte biomass. At both sampling campaigns, temperature, salinity, dissolved oxygen, and pH at the sediment–seawater interface were recorded in triplicate at each site using an HYDROLAB DS5 multiparametric probe.

In the laboratory, after leaf biometry estimation, freeze-dried leaves (both adult and intermediate) from each *P. oceanica* shoot were ground, homogenized, and packed into tin capsules for stable sulfur isotope analysis. Samples were then analyzed using a Thermo IRMS (Delta V ADVANTAGE) coupled with an elemental analyzer (EA Flash 2000). Results are given in the δ notation as per mil deviation from the international standard (V-CDT) as follows: $\delta^{34}\text{S} = [({}^{34}\text{S}/{}^{32}\text{S})_{\text{sample}}/({}^{34}\text{S}/{}^{32}\text{S})_{\text{standard}} - 1] \times 10^3$.

The analytical precision, based on the standard deviation of replicates of internal standards (IAEA-SO-6, IAEA-S-1, NBS 127) was $\pm 0.2\text{‰}$.

Seawater variables and meadow descriptors were analyzed using the following design: Area (two levels: vent, reference) and Year (two levels: 2010, 2011) as orthogonal and fixed factors, and Site as a random factor (nested in both Area and Year). Shoot and leaf descriptors, only available for 2011, were tested considering only the factors Area and Site (nested in Area). Univariate ANOVA was performed on seawater and seagrass descriptors at all levels (meadow, shoot, leaf) and on $\delta^{34}\text{S}$ (STATISTICA 12, StatSoft). The normality assumption was

tested using the Shapiro–Wilk test and the $\log(x + 1)$ transformation was applied when the normality assumption did not hold. Homogeneity of variances was tested using Cochran's test. Multivariate permutational ANOVA (PERMANOVA; PRIMER 6 and PERMANOVA+ package) was run on the seagrass descriptors grouped by level of organization. PERMANOVA was run on the Euclidean distance matrices obtained from $\log(x + 1)$ transformed normalized data, and the Monte-Carlo test was run in the case of low permutations (< 100). Pairwise post hoc tests were conducted when significant effects of the interactions of the factors Area and Year were found.

Results

Seawater variables showed similar values between areas, years, and sites except for pH, which was significantly lower at the vent than at the reference area (Supporting Information Table S1; Signa et al. 2023). At the meadow level, the density of *P. oceanica* was significantly lower at the vent than at the reference area, with no inter-annual or inter-site variability (Fig. 1; Table 1; Supporting Information Table S2).

At the shoot level, significant differences were detected between areas and sites (Supporting Information Table S2). As shown in Table 1, shoots at the vent area were characterized by a higher number of leaves for all age categories (i.e., adult, intermediate and juvenile leaves) together with a higher percentage of brown tissue than at the reference area. On the other hand, shoots showed higher leaf surface and biomass, epiphyte biomass, percentage of green tissue and eroded apices (Coefficient A) in the reference area (Table 1; Supporting Information Table S2). There were no juvenile leaves in the shoots at the reference area (Table 1).

At the leaf level, the differences between the two areas became more pronounced, while the inter-site variability disappeared. Specifically, the values of all descriptors of both adult and intermediate leaves were lower at the vent area than at the reference area (Table 1; Supporting Information Table S2). Similarly, $\delta^{34}\text{S}$ values of *P. oceanica* leaves were significantly lower at the vent than at the reference area, with no difference between sites (Table 1; Supporting Information Table S2).

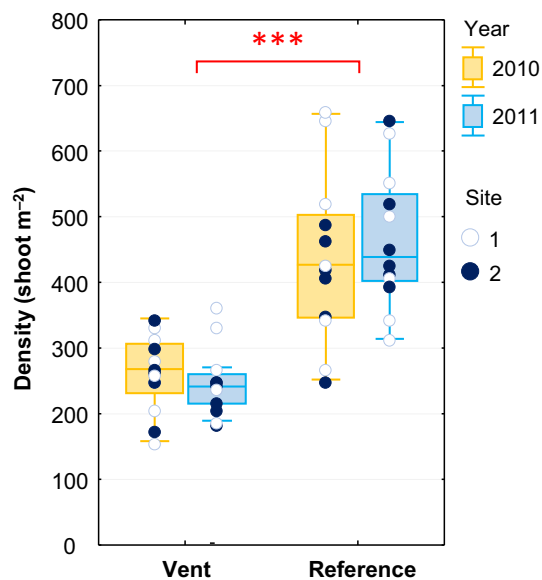


Fig. 1. Density of *Posidonia oceanica* at the two sites of the vent and reference areas in 2010 and 2011 ($n = 12$). Whiskers indicate the non-outlier range of variation; boxes: 25th to 75th percentiles. Significant differences between areas are indicated (see Supporting Information Table S2 for details).

Discussion

This study showed significant impairment in meadow structure and plant phenology of *P. oceanica* living near a shallow volcanic vent affected by both CO₂ and H₂S inputs compared to a reference area. Although the peculiarities of the vent area did not allow to investigate separately the effects of CO₂ and toxic H₂S emissions, the changes in seagrass traits (i.e., reduced density, biomass, canopy height and leaf size), together with the evidence of sulfide intrusion, suggest that CO₂ and H₂S may have acted antagonistically, dampening the

expected CO₂-resource positive effect and resulting in an overall negative response of the plant at the vent area.

Hydrogen sulfide concentration in the vent area was not measured in this study, but the presence of white mat-forming bacteria alongside the typical sulfur smell confirms the presence of this toxic gas, as previously evidenced (Italiano and Nuccio 1991; Caracausi et al. 2005). Furthermore, the decrease in δ³⁴S of *P. oceanica* leaves from the vent area compared to the reference area is a valuable indication not only of the presence of sulfides in the former (Italiano and Nuccio 1991; Steinbrückner 2009; Romano et al. 2019), but also of sulfide intrusion into plant tissues (Holmer and Hasler-Sheetal 2014). Hydrothermal and biogenic porewater sulfides, which are abundant in Panarea sediments (Peters et al. 2011), are thought to be an important source of sulfur in leaf tissues near vent sites, as they enter through roots and are transferred to rhizomes and leaves, when present (Frederiksen et al. 2008). As porewater sulfides typically have negative δ³⁴S values, a decrease in δ³⁴S in seagrass tissues is a valuable proxy for sulfide exposure and intrusion (Holmer and Hasler-Sheetal 2014). Indeed, δ³⁴S of *P. oceanica* leaves from the reference area falls within the range (+15–27‰) reported by Holmer and Hasler-Sheetal (2014) for areas unaffected by sulfides, while the decrease of about 5‰ found near the Panarea vents provides evidence that some amount of sulfur from sedimentary sulfides has accumulated in *P. oceanica* leaves. Furthermore, the reduction in both leaf δ³⁴S and biometric parameters provides clear evidence of the stressor effect of sulfide intrusion on seagrass performance in line with previous studies showing reduced growth, meristem activity and above-ground productivity and increased mortality following sulfide intrusion in *P. oceanica* (Calleja et al. 2007; Frederiksen et al. 2008; Garcias-Bonet et al. 2008).

The reduction in shoot density and biomass found here was opposite to previous findings on *P. oceanica* living near the volcanic vent of Ischia (Italy, Tyrrhenian Sea) where volcanic gas emissions are constituted by CO₂ with no hydrogen sulfide (Foo et al. 2018). Under these “pure” acidic conditions, *P. oceanica* thrives at densities twice as high as those observed in the reference area and 10-fold higher than those found in the vent area of the present study (Hall-Spencer et al. 2008; Mecca et al. 2020; Mirasole et al. 2021). In contrast, patterns similar to those found in this study were highlighted for *Cymodocea nodosa* growing nearby Vulcano vents (Eolian Archipelago) (Apostolaki et al. 2014), where the seagrass is likely to be stressed by H₂S, CH₄, and metals (Capaccioni et al. 2001; Vizzini et al. 2013). The contrasting characteristics of gas emissions at Panarea and Ischia vents may also account for the different expression patterns of key stress-related genes previously observed in *P. oceanica* near the two vent systems, representing the result of site-specific environmental stress rather than the reflection of contrasting homeostatic evolutionary compensation (Lauritano et al. 2015).

As regards plant phenology, it is worth noting that the leaves of the shoots at the vent area were more numerous than at reference conditions, but they were also shorter and thinner,

with a higher proportion of brown than green tissue. Changes in leaf color may be the result of (i) the alteration of the ratio between phaeopigments and chlorophyll *a*, as already observed in *Zostera noltii* under thermal stress (i.e., warming) (Repolho et al. 2017) and/or (ii) a faster leaf aging process under sulfide stress (Lamers et al. 2013). This is further supported by the presence of young leaves only in plants close to the vents, consistent with increased leaf turnover (Perry et al. 2019) probably as a physiological mechanism to offset the early leaf aging.

The undersizing of *P. oceanica* leaves in response to extreme environmental conditions has already been observed in Mediterranean marine areas (Gravili et al. 2021; Mancuso et al. 2023; Nguyen et al. 2023), including Panarea, where *P. oceanica* “bonsai” shoots have recently been described (Gambi et al. 2023). Consistently, we found smaller plants near the vents, but with more leaves than at reference sites, confirming the complexity of the plant response to multiple gases and the need for further investigation, especially to distinguish acclimatization mechanisms from young plants recovering after strong disturbances (parossistic events). The divergent pattern found here between high leaf abundance and turnover and low leaf size at the vent area seems to mirror the occurrence of acclimatization mechanisms in *P. oceanica* exposed to combined CO₂ and H₂S emissions, resulting in a scarce efficiency of the plant to allocate the additional carbon of volcanic origin into new biomass under stressful conditions (Invers et al. 2001; Apostolaki et al. 2014; Vizzini et al. 2019), rather than a higher grazing pressure. Indeed, the low Coefficient A (i.e., number of eroded leaf apices) found in the vents confirms this hypothesis, suggesting low exploitation of *P. oceanica* by herbivores and macrograzers, contrary to previous observations in the “pure” CO₂ Ischia vents (Donnarumma et al. 2014; Mirasole et al. 2020). Indeed, OA is commonly associated with increased nutritional value (low C : N ratio) and decreased deterrent compounds (i.e., phenolics) in *P. oceanica* and, consequently, higher seagrass exploitation by herbivores (Ricevuto et al. 2015), all aspects that deserve further research under the complex environmental conditions of the Panarea vent system.

Differently, the drastic reduction in epiphyte biomass observed near the vents is consistent with the classic response of calcifying epibionts to OA (Hall-Spencer et al. 2008; Nogueira et al. 2017), although the low leaf size and faster leaf turnover may have certainly played a role, resulting in a reduced leaf surface and time available for epiphyte colonization. The low epiphyte colonization observed in the vent area may also have increased light exposure, which in turn may have contributed to the previously discussed leaf aging process and die-off (brown tissue) (Munné-Bosch and Alegre 2002; Zimmermann and Zentgraf 2005).

Overall, the outcomes of this study underlined highly stressful conditions associated with sulfide intrusion in the tissues of *P. oceanica* living near the Panarea vents and the consequent negative response in plant density and biomass. This response contrasts with the thriving condition of the same

species previously described at the Ischia vents, where there is no evidence of toxic inputs (Hall-Spencer et al. 2008; Mecca et al. 2020; Mirasole et al. 2021), suggesting that H₂S emissions from volcanic vents, when they occur, affect seagrass performance, net of the potential beneficial effects of increasing CO₂ concentrations. Indeed, we observed here that the response of seagrasses to OA can deviate from expectations when increased CO₂ is combined with chemical stressors, and that this response is as complex as highly context-dependent, given the variability of volcanic input at shallow vents (Koch et al. 2013; Mostofa et al. 2016; Sunday et al. 2017; Collier et al. 2018; Hall-Spencer and Harvey 2019; Perry et al. 2019). Moreover, we revealed that eco-physiological mechanisms of acclimatization occur at multiple levels (from leaf to meadow) of *P. oceanica* long-term exposed to CO₂ and toxicants, paving the way for further research on the response of seagrass ecosystems under future global change scenarios. Although the results of this study suggest that the combination of CO₂ and H₂S may act antagonistically on *P. oceanica*, further studies are needed to determine the specific underlying mechanisms, using specific multiple stressor designs and possibly combining field and manipulative approaches.

Data availability statement

Data and metadata are available on Zenodo at <https://zenodo.org/records/8425339>.

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